



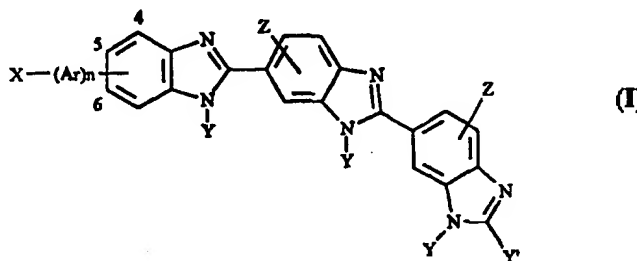
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<p>(21) International Application Number: PCT/US96/06853</p> <p>(22) International Filing Date: 14 May 1996 (14.05.96)</p> <p>(30) Priority Data:</p> <table border="0"> <tr> <td>08/442,657</td> <td>17 May 1995 (17.05.95)</td> <td>US</td> </tr> <tr> <td>08/618,988</td> <td>20 March 1996 (20.03.96)</td> <td>US</td> </tr> </table> <p>(71) Applicant (for all designated States except US): RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY [US/US]; Old Queens Building, Somerset and George Streets, New Brunswick, NJ 08903 (US).</p> <p>(71)(72) Applicants and Inventors: LAVOIE, Edmond, J. [US/US]; 3 Guilford Court, Princeton Junction, NJ 08550 (US). LIU, Leroy, Fong [US/US]; 5 Fairacres Drive, Bridgewater, NJ 08807 (US). SUN, Qun [CN/CN]; Apartment 22-201, ReMian Road, Xia Shan, Zhejiang 311200 (CN).</p> <p>(74) Agent: VIKSNINS, Ann, S.; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).</p>		08/442,657	17 May 1995 (17.05.95)	US	08/618,988	20 March 1996 (20.03.96)	US	<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>
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(54) Title: TRIBENZIMIDAZOLES USEFUL AS TOPOISOMERASE I INHIBITORS

(57) Abstract

The present invention provides anti-neoplastic topoisomerase I inhibitors of formula (I) wherein Ar is (C₆-C₁₂)aryl or (5- to 12-membered) heteroaryl comprising 1-3 N, S or non peroxy O, wherein N is unsubstituted or is substituted with (C₁-C₄)alkyl; X is H, CN, CHO, OH, acetyl, CF₃, O(C₁-C₄)alkyl, NO₂, NH₂, halogen or halo-(C₁-C₄)alkyl; each Y is individually H, (C₁-C₄)alkyl or aralkyl; Y' is H or (C₁-C₄)alkyl; n is 0 or 1; and each Z is individually H, (C₁-C₄)alkyl, halogen or halo-(C₁-C₄)alkyl; or a pharmaceutically acceptable salt thereof.



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TRISBENZIMIDAZOLES USEFUL AS TOPOISOMERASE I INHIBITORS

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Background of the Invention

DNA topoisomerases are nuclear enzymes that control and modify the topological states of DNA by catalyzing the concerted breaking and rejoining of DNA strands. See, for example, D'Arpa et al., Biochim. Biophys. Acta, **989**, 163 (1989). Topoisomerase II enzymes alter the topological state of DNA by means of a double strand break in the DNA. Mammalian topoisomerase II represents an effective pharmacological target for the development of cancer chemotherapeutics. (A. Y. Chen et al., Annu. Rev. Pharmacol. Toxicol., **34**, 191 (1994)). Among the clinical agents in use which are recognized as topoisomerase II inhibitors are etoposide (VP-16), teniposide (VM-26), mitoxantrone, *m*-AMSA, adriamycin (doxorubicin), ellipticine and daunomycin.

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In comparison to topoisomerase II inhibitors, there are relatively few known topoisomerase I inhibitors. Camptothecin represents the most extensively studied mammalian topoisomerase I inhibitor. See R. C. Gallo et al., J. Natl. Cancer Inst., **46**, 789 (1971) and B. C. Giovanella et al., Cancer Res., **51**, 3052 (1991). The broad spectrum of potent antineoplastic activity observed for camptothecin has prompted further efforts to identify other agents which can effectively poison mammalian topoisomerase I.

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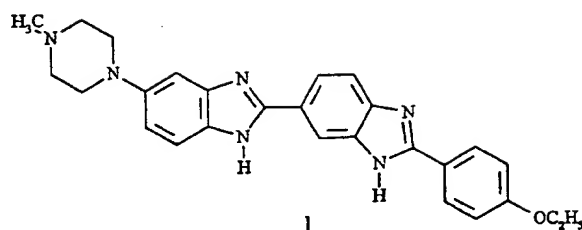
It has recently been demonstrated that Hoechst 33342 (1), 2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole, is an inhibitor of topoisomerase I.

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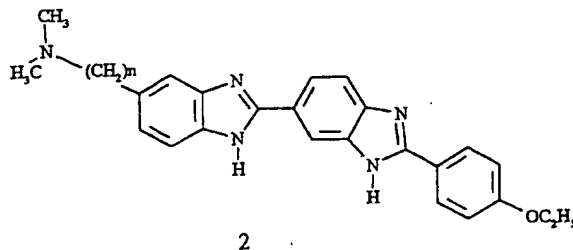
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This agent, which binds to the minor groove of DNA, traps the reversible cleavable complex derived from DNA and topoisomerase I and produces a limited number of highly specific single-strand DNA breaks. For example, see A.Y. Chen et al., Cancer Res., **53**, 1332 (1993) and A. Chen et al., PNAS, **90**, 8131 (1993). A limitation of Hoechst 33342 as an anticancer agent is the previously reported observation that it is not effective against tumor cell lines which overexpress MDR1. While KB 3-1 cells are known to be quite sensitive to Hoechst 33342, with an IC_{50} of approximately 9 nM, this compound is approximately 130-fold less cytotoxic to KB V-1 cells, which are known to overexpress MDR1. Recently, several analogs of this bisbenzimidazole have been synthesized, to further investigate the structure activity relationships associated with their potency as topoisomerase I inhibitors and the related cytotoxicity. For example, Q. Sun et al., Biorg. and Med. Chem. Lett., **4**, 2871 (1994) disclosed the preparation of bisbenzimidazoles of formula (2):

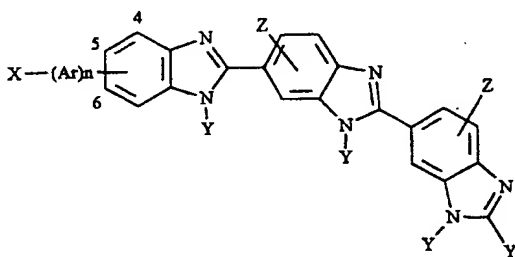
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where n is 0, 1, 2, or 3. However, these compounds were found to be about one order of magnitude less cytotoxic than Hoechst 33342. Therefore, a continuing need exists for new compounds that can induce DNA cleavage in the presence of mammalian topoisomerase I.

Summary of the Invention

The present invention provides a compound of general formula (I):



wherein Ar is aryl or a nitrogen-, sulfur- or oxygen-containing heteroaromatic group; X is H, CN, CHO, OH, acetyl, CF₃, O(C₁-C₄)alkyl, NO₂, NH₂, halogen or halo(C₁-C₄)alkyl; each Y is individually H, (C₁-C₄)alkyl or aralkyl; Y' is H or (C₁-C₄)alkyl; each Z is individually H, (C₁-C₄)alkyl, halogen or halo(C₁-C₄)alkyl; and n is 0 or 1; or a pharmaceutically acceptable salt thereof. Preferably, Ar is a (C₆-C₁₂)aryl or a 5- to 12-membered heteroaryl group comprising 1-3 N, S or non-peroxide O atoms in the ring, wherein each N is unsubstituted or is substituted with (C₁-C₄)alkyl. As drawn, the Ar-group can occupy any position of the benzo moiety, i.e., positions 4-7, preferably the 5 position, and X can occupy any available position on Ar. The Ar-group may be optionally fused to the benzo moiety, preferably at the 4, 5-, or 5, 6- positions. In one preferred embodiment, Ar is phenyl, and X is H or is a 4-substituent. According to another preferred embodiment, Ar is phenyl, and X is Cl or Br, preferably occupying the *para*

position. As drawn, Z may occupy any position on the benzo moiety. Z is preferably H, halogen, CH₃ or CF₃.

According to another embodiment, n is 0, and X is preferably H, CN, CHO or halogen, for example, F, Br, Cl or I, preferably Cl or Br, and preferably occupies the 5-position of the benzo moiety. Y is preferably H or CH₃. Y' is preferably H or CH₃.

Compounds of formula (I) are inhibitors of topoisomerase I, as demonstrated by their ability to promote DNA cleavage in the presence of topoisomerase I. Furthermore, compounds of formula (I) also are cytotoxic to mammalian tumor cells, including camptothecin-sensitive and camptothecin-resistant tumor cells and tumor cell lines exhibiting multi-drug resistance due to expression of the P-glycoprotein.

Therefore, the present invention also provides a method for the inhibition of mammalian tumor cell growth, comprising contacting a susceptible population of tumor cells with an effective growth-inhibiting amount of a compound of formula (I), preferably in combination with a pharmaceutically acceptable carrier. The growth of the tumor cells can be inhibited in vitro, or vivo, by administering the compound of formula (I) to a mammal in need of such treatment, such as a human cancer patient afflicted with a leukemia or solid tumor. The compounds of formula I can also be used to evaluate the activity of topoisomerase I obtained from different sources, and are expected to exhibit at least some of the other bioactivities observed for topoisomerase inhibitors, such as antibacterial, antifungal, antiprotozoal, anthelmintic and/or antiviral activity. For example, compound 14, shown on Figure 1, exhibits antifungal activity.

Brief Description of the Drawings

Figure 1 is a schematic depiction of the synthesis of compounds 10-16.

Figure 2 is a schematic depiction of the preparation of intermediates 4-8 used to prepare compounds of the invention.

Figure 3 is a schematic depiction of the preparation of intermediate 2.

Figure 4 is a schematic depiction of the synthesis of compounds JSKIV-68, -37 and -47.

Figure 5 is a schematic depiction of the preparation of intermediate JSKIV-44.

Figure 6 is a schematic depiction of the preparation of analogs modified on the central benzimidazole moiety.

Figure 7 is a schematic depiction of the preparation of analogs modified on the terminal benzimidazole moiety.

Detailed Description of the Invention

The aryl groups (Ar) useful in the present compounds comprise (C₆-C₁₈)aryl, preferably (C₆-C₁₄) aryl, e.g., systems containing aromatic rings, which systems comprise a total of 6 to 12 carbon atoms. Thus, as used herein, the term "aryl" includes mono- or bis-(C₁-C₄)alkyl-substituted aryl, such as tolyl and xylyl; ar(C₁-C₄)alkyl, such as benzyl or phenethyl; and alkaralkyl. Preferably aryl is phenyl, benzyl or naphthyl.

Heteroaromatic rings include aromatic rings containing up to 3 ring heteroatoms such as N, S or non-peroxide O, and up to 12 ring atoms.

Representative aromatic rings include thiophene, benzothiophene, naphthothiophene, trianthrene, furan, benzofuran, isobenzofuran, pyran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, pyridine, pyrazine, triazole, tetrazole, pyrazine, triazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, phenazine, isothiazole, phenothiazine, oxazole, isoxazole, furazan, phenoxazine and the like. Preferred heteroaromatic rings have a 5- or 6-membered heteroaromatic ring which may or may not be fused to an aromatic ring such as a benzo ring, e.g., the preferred 2-, 3- or 4-pyridyl substituents.

The term "alkyl" includes straight-chain or branched alkyl, as well as cycloalkyl and (cyloalkyl)alkyl, e.g., methyl, ethyl, i-propyl, cyclopropyl or cyclopropylmethyl.

Pharmaceutically acceptable salts include the acid addition salts of basic NH with organic or inorganic acids, e.g., hydrochloride, carbonate, sulfate, acetate, phosphate, tartarate, citrate, malate, maleate, propionate, and the like.

The preparation of representative substituted trisbenzimidazoles is outlined in Figure 1. With the exception of phenylenediamine which was commercially available, the appropriately substituted phenylenediamines were synthesized by catalytic hydrogenation of the respective *o*-nitroaniline derivatives. These phenylenediamines were then coupled with 5-formyl-2-(benzimidazo-5'-yl)benzimidazole, **2**, by heating in nitrobenzene at 150°C to provide the various trisbenzimidazoles, **10-16**, in yields ranging from 43-96%, employing the general methodologies of M. P. Singh et al., Chem. Res. Toxicol., **5**, 597 (1992) and Y. Bathini et al., Synth Comm., **20**, 955 (1990).

The requisite nitroanilines, as outlined in Figure 1, with the exception of **3** which was commercially available, were synthesized from 4-bromo-2-nitroaniline, **17**. Compound **17** was prepared from *o*-nitroaniline in good yield, 94%, using 2,4,4,6-tetrabromo-2,5-cyclohexadienone as the bromination reagent. G. J. Fox et al., Org. Syn., **55**, 20 (1973). While allyltributyltin and phenyltributyltin are commercially available, the pyridyltributyltin derivatives were prepared from tributyltin chloride and 2-, 3-, and 4-bromopyridine, respectively. See D. Peters et al., Heterocyclic Chem., **27**, 2165 (1990). These tributyltin derivatives were then coupled with 4-bromo-2-nitroaniline using PdCl₂(PPh₃)₂ as the catalyst in DMF as outlined in Figure 2 to provide compounds **4**, **5**, **6**, **7**, and **8**, respectively, in accord with the methodology of M. Iwao et al., Heterocycles, **36**, 1483 (1993). This methodology can generally be applied to prepare 3-, 4-, 5- or 6-aryl- and heteroaryl-substituted 2-nitroanilines from the corresponding bromonitroanilines.

The preparation of 5-formyl-2-(benzimidazo-5'-yl)benzimidazole, 9, was accomplished as outlined in Figure 3. Reduction of 5-benzimidazolecarboxylic acid to 5-hydroxymethylbenzimidazole was accomplished using LiAlH_4 . Oxidation of the resulting crude benzylic alcohol with tetrapropylammonium perruthenate (TPAP) and N-methylmorpholine N-oxide provided in two steps the desired 5-formylbenzimidazole in 32% an overall yield. See, A. Cherif et al., J. Med. Chem., 35, 3208 (1992). Coupling of 5-formylbenzimidazole with 4-cyano-1,2-phenylenediamine provided 5-cyano-2-(benzimidazol-5'-yl)benzimidazole, 19, which when treated with Ni-Al catalyst in the presence of aqueous formic acid gave 5-formyl-2-(benzimidazol-5'-yl)benzimidazole, 9, in 65% yield. (J. R. Pipier et al., J. Med. Chem., 31, 2164 (1988)).

The compounds of the present invention can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human cancer patient, in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenously, intramuscularly or subcutaneous routes.

Thus, the present phosphoramidate compounds may be orally administered, for example, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion use can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier

or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersion or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

Useful dosages of the compounds of 1 can be determined by comparing their in vitro activity, and in vivo activity in animal models, to that of an equivalent dosage of camptothecin (see, for example, B. C. Giovanella et al., Cancer Res., 51, 3052 (1991)) or Hoechst 33342 (see, A. Y. Chen et al., Cancer Res., 53, 1332 (1993)). Methods for the extrapolation of effective anti-tumor dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

The present analogs can be used to treat cancers known to be susceptible to topoisomerase I inhibitors, including, but not limited to, Burkitt's tumor, chronic lymphocytic leukemia, multiple myeloma, squamous cell and large

cell anaplastic carcinomas, adenocarcinoma of the lung, Ewing's sarcoma, non-Hodgkins lymphoma, breast tumor, colon tumor, stomach tumor, oat-cell bronchogenic carcinoma, squamous cell carcinoma of the cervix, ovarian tumors, bladder tumors, testicular tumors, endometrial tumors, malignant melanoma and acute lymphocytic leukemia, and prostatic carcinoma. The present compounds can be administered as single agents, or in combination with other antineoplastic drugs commonly employed to treat these cancers.

The invention will be further described by reference to the following detailed examples, wherein melting points were determined with a Thomas-Hoover unimelt capillary melting point apparatus. Infrared spectral data (IR) were obtained on a Perkin-Elmer 1600 Fourier transform spectrophotometer and are reported in cm^{-1} . Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in CDCl_3 (unless otherwise noted) with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz. Mass spectra were obtained from Midwest Center for Mass Spectrometry within the Department of Chemistry at the University of Nebraska-Lincoln. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within $\pm 0.4\%$. THF was freshly distilled from sodium and benzophenone prior to use. Allyltributyltin and phenyltributyltin were purchased from Aldrich Chemical Company.

Example 1. General Procedure for $\text{PdCl}_2(\text{PPh}_3)_2$ -catalyzed Coupling Reaction of 4-Bromo-2-nitroaniline (13) with Tin Compounds.

(A) 4-Phenyl-2-nitroaniline (5). A solution of 4-bromo-2-nitroaniline (1.0 g, 4.67 mmol), tributylphenyl tin (2.2 g, 6.07 mmol), bis(triphenylphosphine)palladium (II) chloride (164 mg, 0.234 mmol), and triphenylphosphine (613 mg, 2.34 mmol) in DMF (15 ml) was heated under N_2 at 120°C overnight. After the solution was cooled to room temperature, the reaction

mixture was directly chromatographed on silica gel eluting with 2-5%

EtOAc/Hexane to give 752 mg (75%) of **5** as a yellow solid: mp 169-171 °C; IR (CHCl₃) 3517, 3398, 3022, 1635, 1525, 1250; ¹H NMR δ 8.38 (1H, d, *J* = 2.2), 7.66 (1H, dd, *J* = 8.7, 2.2), 7.59-7.54 (2H, m), 7.49-7.34 (3H, m), 6.90 (1H, d, *J* = 8.8), 6.13 (NH, brs); ¹³C NMR δ 144.2, 139.3, 135.0, 130.9, 129.5, 127.8, 126.8, 124.4, 119.8, 112.8; Anal. Calcd for C₁₂H₁₀N₂O₂: C, 67.28; H, 4.70; N, 13.08. Found: C, 67.38, H, 4.76; N, 13.01.

(B) 4-Allyl-2-nitroaniline (4). Prepared from 4-bromo-2-nitroaniline **17** (1.70 g, 7.84 mmol) and allyltributyltin (3.38 g, 10.2 mmol) as a yellow solid in 96% yield as described above for **5**: mp 29-31 °C; IR (KBr) 3490, 3374, 1638, 1518, 1341, 1253; ¹H NMR δ 7.90 (1H, d, *J* = 2.0), 7.19 (1H, dd, *J* = 8.5, 2.0), 6.77 (1H, d, *J* = 8.5), 6.05 (NH, brs), 6.00-5.80 (1H, m), 5.11 (1H, dd, *J* = 1.4, 1.4), 5.04 (1H, ddd, *J* = 6.6, 3.0, 1.5), 3.28 (1H, d, *J* = 6.6); ¹³C NMR δ 143.81, 137.13, 129.34, 125.59, 119.49, 116.95, 39.18; HRMS (EI) calcd for C₉H₁₀N₂O₂ 178.0742, found 178.0746.

(C) 4-(2'-Pyridyl)-2-nitroaniline (6). Prepared from 4-bromo-2-nitroaniline **17** (597 mg, 2.75 mmol) and 2-tributylstannylpyridine (1.01 g, 2.75 mmol) as a yellow solid in 52% yield as described above for **5**: mp 146-148 °C; IR (CHCl₃) 3516, 3397, 3020, 1634, 1524, 1341, 1250; ¹H NMR δ 8.74 (1H, d, *J* = 2.2), 8.63 (1H, dd, *J* = 4.9, 1.5), 8.13 (1H, dd, *J* = 8.8, 2.1), 7.78-7.66 (2H, m), 7.20 (1H, ddd, *J* = 4.8, 4.7, 1.9), 6.92 (1H, d, *J* = 8.8), 6.37 (NH, brs); ¹³C NMR δ 155.6, 150.1, 145.6, 137.4, 134.5, 129.1, 124.7, 122.4, 119.8, 119.7; Anal. Calcd for C₁₁H₉N₃O₂: C, 61.39; H, 4.21; N, 19.53. Found: C, 61.29; H, 4.23; N, 19.43.

(D) 4-(3'-Pyridyl)-2-nitroaniline (7). Prepared from 4-bromo-2-nitroaniline **17** (1.42 g, 6.53 mmol) and 3-tributylstannylpyridine (3.60 g, 9.79 mmol) as a yellow solid in 32% yield as described above for **5**: mp 177-179 °C; IR (CHCl₃) 3515, 3399, 3052, 2983, 1638, 1524, 1341, 1259; ¹H NMR δ 8.68 (1H, d, *J* = 1.7), 8.42 (1H, dd, *J* = 4.8, 1.5), 8.22 (1H, d, *J* = 2.2), 7.74 (1H, ddd, *J* = 7.9, 2.4, 1.6), 7.50 (1H, dd, *J* = 8.7, 2.2), 7.23 (1H, ddd, *J* = 8.0, 4.8, 0.8), 6.92 (1H, d, *J* =

8.8), 6.56 (NH, brs); ^{13}C NMR δ 148.7, 147.8, 145.4, 135.0, 134.4, 133.8, 126.5, 124.4, 124.0, 120.4; Anal. Calcd for $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_2$: C, 61.39; H, 4.21; N, 19.53. Found: C, 61.28; H, 4.16; N, 19.40.

(E) 4-(4'-Pyridyl)-2-nitroaniline (8). Prepared from 4-bromo-2-nitroaniline 17 (165 mg, 0.76 mmol) and 4-tributylstannylpyridine (280 mg, 0.76 mmol) as a yellow solid in 25% yield as described above for 5: mp 230-232 °C; IR (CHCl₃) 3518, 3398, 3032, 1636, 1528, 1344; ^1H NMR (CD₃OD) δ 8.55 (2H, d, J = 6.3), 8.52 (1H, d, J = 2.3), 7.84 (1H, dd, J = 8.9, 2.3), 7.71 (2H, d, J = 6.4), 7.13 (1H, d, J = 8.9); ^{13}C NMR (CD₃OD) δ 149.4, 133.4, 124.0, 120.7, 120.0; HRMS (EI) calcd for $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_2$ 215.0695, found 215.0698.

Example 2. 5-Formyl-2-(benzimidazol-5'-yl)benzimidazole (9).

A mixture of 5-cyano-2-(benzimidazol-5'-yl)benzimidazole 19 (148 mg, 0.57 mmol), Ni-Al catalyst (500 mg), formic acid (7 ml) and water (3 ml) was heated under reflux under N₂ for 4h. The hot reaction mixture was immediately filtered through a plug of celite, and evaporated to give a yellow solid. The yellow solid was then dissolved in hot water (5 ml), and the solution was neutralized to pH 9 by 2N NaOH. The solid precipitated was collected by suction filtration and further purified by flash chromatography on silica gel (15% MeOH/EtOAc) to give 142 mg (95%) of 9 as a white solid: mp > 275 °C; IR (KBr) 3106, 2835, 1685, 1618, 1432, 1293; ^1H NMR (CD₃OD) δ 10.01 (1H, s), 8.39 (1H, s), 8.35 (1H, s), 8.13 (1H, s), 8.06 (1H, dd, J = 8.6, 1.6), 7.83 (1H, dd, J = 8.4, 1.4), 7.77 (1H, d, J = 8.5), 7.71 (1H, d, J = 8.3); HRMS (FAB) calcd for $\text{C}_{15}\text{H}_{11}\text{N}_4\text{O}$ 263.0933, found 263.0932.

Example 3. General Procedures for Preparing 5-substituted trisbenzimidazoles.

(A) 2-[2'-(Benzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (10).

A mixture of 5-formyl-2-(benzimidazol-5'-yl)benzimidazole 9 (121 mg, 0.46 mmol) and phenylenediamine (60 mg, 0.55 mmol) in nitrobenzene (8 ml) was

heated at 150 °C under N₂ overnight. The mixture was cooled to room temperature and chromatographed on silica gel (0-20% MeOH/EtOAc) to afford 155 mg (96%) of **10** as a solid: mp > 275 °C; IR (KBr) 3400, 3157, 1630, 1542, 1438, 1294; ¹H NMR (DMSO-d₆ + 3 drops of CF₃COOH) δ 9.71 (1H, s), 8.75 (1H, s), 8.65 (1H, d, J = 1.1), 8.48 (1H, dd, J = 8.7, 1.5), 8.21 (1H, dd, J = 8.6, 1.6), 8.14 (1H, d, J = 8.8), 8.08 (1H, d, J = 8.7), 7.90 (2H, dd, J = 6.2, 3.1), 7.61 (2H, dd, J = 6.1, 3.1); ¹³C NMR (DMSO-d₆ + 3 drops of CF₃COOH) δ 154.4, 149.8, 133.2, 132.0, 131.7, 126.2, 125.5, 125.4, 123.9, 123.6, 116.3, 115.9, 114.23, 114.17, 114.13; HRMS (FAB) calcd for C₂₁H₁₅N₆ 351.1358, found 351.1367.

(B) 5-Cyano-2-[2'-(benzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (11). Hydrogenation of **3** (70 mg, 0.43 mmol) was accomplished at 40 psi H₂ at room temperature for 1 h using 10% Pd-C (30 mg) in EtOAc (10 ml). The reaction mixture was filtered and concentrated *in vacuo* to afford a solid. The solution of this solid and **9** (87 mg, 0.33 mmol) in nitrobenzene (5 ml) was heated at 150 °C under N₂ overnight. The mixture was cooled to room temperature, and chromatographed directly on silica gel (0-10% MeOH/EtOAc) to give 107 mg (86%) of **11** as a solid; mp > 280 °C; IR (KBr) 3416, 3148, 2222, 1626, 1553, 1441, 1292; ¹H NMR (DMSO-d₆ + 3 drops of CF₃COOH) δ 8.50 (1H, s), 8.46 (1H, s), 8.40 (1H, s), 8.18-8.11 (3H, m), 7.81-7.75 (3H, m), 7.62 (1H, dd, J = 8.3, 1.5); HRMS (FAB) calcd for C₂₂H₁₃N₇ 376.1310, found 376.1309.

(C) 5-Propyl-2-[2'-(benzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (12). Prepared from 4-allyl-2-nitroaniline **4** (312 mg, 1.75 mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole **9** (121 mg, 0.46 mmol) in 79% yield as described above for **11**: solid; mp > 270 °C; IR (KBr) 3421, 3068, 2957, 1434; ¹H NMR (DMSO-d₆ + 3 drops of CF₃COOH) δ 9.66 (1H, s), 8.73 (1H, s), 8.59 (1H, s), 8.48 (1H, dd, J = 8.7, 1.5), 8.13 (1H, dd, J = 8.7, 1.4), 8.11 (1H, d, J = 8.7), 8.02 (1H, d, J = 8.5), 7.79 (1H, d, J = 8.4), 7.66 (1H, s), 7.45 (1H, dd, J = 8.5, 1.3), 2.80 (2H, t, J = 7.0), 1.70 (2H, m), 0.96 (3H, t, J = 7.2); ¹³C NMR (DMSO-d₆ + 3 drops of CF₃COOH) δ 153.84, 149.74, 141.64, 141.01, 139.37,

133.10, 132.26, 131.99, 130.34, 127.08, 126.26, 125.14, 141.64, 141.01, 139.37,
133.10, 132.26, 131.99, 130.34, 127.08, 126.26, 125.14, 122.91, 117.52, 116.32,
116.06, 115.76, 113.78, 112.99, 37.45, 24.73, 13.74;

(D) 5-Phenyl-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-

5 **yl]benzimidazole (13).** Prepared from 4-phenyl-2-nitroaniline 5 (247 mg, 1.15
mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole 9 (201 mg, 0.77 mmol)
in 89% yield as described for 11: solid; mp 262-164 °C dec; IR (KBr) 3402, 3104,
1627, 1552, 1442, 1290; ¹H NMR (DMSO-d₆ + 3 drops of CF₃COOH) δ 9.66 (1H,
s), 8.74 (1H, s), 8.65 (1H, s), 8.50 (1H, dd, *J* = 8.8, 1.1), 8.21 (1H, dd, *J* = 8.7, 1.4),
10 8.12 (1H, d, *J* = 8.8), 8.06 (1H, s), 8.05 (1H, d, *J* = 8.4), 7.97 (1H, d, *J* = 8.7), 7.89
(1H, dd, *J* = 8.7, 1.5), 7.80 (2H, d, *J* = 7.0), 7.61-7.47 (3H, m); HRMS (FAB) calcd
for C₂₇H₁₉N₆ 427.1671, found 427.1666.

(E) 5-(2-Pyridyl)-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-

15 **yl]benzimidazole (14).** Prepared from 4-(2'-pyridyl)-2-nitroaniline, 6 (110 mg,
0.50 mmol), and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole 9 (51 mg, 0.25
mmol) in 84% yield as described above for 11: solid; mp > 275 °C; IR (KBr) 3411,
3157, 1630, 1593, 1432; ¹H NMR (CD₃OD) δ 8.59 (1H, d, *J* = 4.8), 8.35 (1H, s),
8.31-8.25 (2H, m), 8.10 (1H, s), 8.04-7.94 (2H, m), 7.85-7.77 (3H, m), 7.72 (1H, d,
J = 8.6), 7.68 (1H, d, *J* = 8.7), 7.64 (1H, d, *J* = 8.7), 7.30 (1H, m); HRMS (FAB)
20 calcd for C₂₆H₁₈N₇ 428.1624, found 428.1611.

(F) 5-(3-Pyridyl)-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-

25 **yl]benzimidazole (15).** Prepared from 4-(3'-pyridyl)-2-nitroaniline 7 (183 mg,
0.85 mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole 9 in 46% yield as
described above for 11: solid; mp > 275 °C; IR (KBr) 3400, 3070, 2836, 1438,
1289; ¹H NMR (CD₃OD) δ 8.83 (1H, d, *J* = 1.6), 8.49 (1H, dd, *J* = 4.9, 1.5), 8.38
(1H, d, *J* = 1.1), 8.31 (1H, d, *J* = 1.1), 8.29 (1H, s), 8.11 (1H, ddd, *J* = 8.0, 2.3, 1.6),
8.05 (1H, dd, *J* = 8.5, 1.6), 8.00 (1H, dd, *J* = 8.5, 1.6), 7.81 (1H, d, *J* = 1.1), 7.77-
7.68 (3H, m), 7.55-7.47 (2H, m); HRMS (FAB) calcd for C₂₆H₁₈N₇ 428.1624, found
428.1612.

(G) 5-(4-Pyridyl)-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-yl]benzimidazole (16). Prepared from 4-(4'-pyridyl)-2-nitroaniline 8 (35 mg, 0.16 mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole 9 (50 mg, 0.19 mmol) in 43% yield as described above for 11: solid; mp > 280 °C; IR (KBr) 3411, 3118, 1600, 1552, 1439, 1290; ¹H NMR (CD₃OD) δ 8.51 (2H, d, J = 6.2), 8.33 (1H, d, J = 1.1), 8.27 (1H, s), 8.25 (1H, d, J = 1.1), 8.01 (1H, dd, J = 8.6, 1.7), 7.96 (1H, dd, J = 8.9, 2.0), 7.87 (1H, d, J = 1.0), 7.74-7.56 (6H, m); HRMS (FAB) calcd for C₂₆H₁₈N₇, 428.1624, found 428.1625.

10 **Example 4. 4-Bromo-2-nitroaniline (17).**

A solution of 2-nitroaniline (5 g, 36.2 mmol) in CH₂Cl₂ (100 ml) was cooled to -10°C, and treated by 90% 2,4,4,6-tetrabromo-2,5-cyclohexadienone (19.8 g, 43.5 mmol) in 5 portions. The mixture was stirred at -10°C-0°C for 1 hr. After being warmed to room temperature, the reaction mixture was washed by 2N NaOH (60 ml) and brine (50 ml), dried over Na₂SO₄ and evaporated. Flash chromatography on silica gel (5% EtOAc/Hexane) gave 7.40 g (94%) of 17 as a yellow solid: mp 109-110 (lit. mp 112-113 °C); ¹H NMR δ 8.27 (1H, d, J = 2.3), 7.43 (1H, dd, J = 8.9, 2.4), 6.73 (1H, d, J = 8.8), 6.09 (NH, brs).

20 **Example 5. 5-Formylbenzimidazole (18).**

A suspension of 5-benzimidazolecarboxylic acid (1.57 g, 9.7 mmol) in dry THF (50 ml) was cooled to -78°C under N₂, and treated with LiAlH₄ (736 mg, 19.4 mmol). After the addition, the mixture was allowed to warm slowly to room temperature and then stirred at r.t. overnight. The mixture was quenched by MeOH and H₂O cautiously, and passed through a short silica gel column eluting with 10% MeOH/EtOAc. The eluate was concentrated to give 876 mg crude alcohol as a solid. The crude alcohol (876 mg) was dissolved in a mixture of DMF (3 ml), THF (10 ml) and CH₂Cl₂ (40 ml). 4-Methylmorpholine N-oxide (2.25 g, 19.2 mmol), 4A molecular sieves (5 g), and TPAP (169 mg, 0.48 mmol) were subsequently added to

the crude alcohol solution. The mixture was stirred at room temperature overnight, and filtered through a pad of silica gel eluting with 10% MeOH/EtOAc. The elute was concentrated and further purified by flash chromatography on silica gel eluting with 0-10% MeOH/EtOAc to give 452 mg (32%, 2 steps) of 17 as a white solid: mp 164-166 °C; IR (KBr) 3087, 2818, 1690, 1292; ¹H NMR (CD₃OD) δ 9.95 (1H, s), 8.34 (1H, s), 8.08 (1H, d, *J* = 1.5), 7.74 (1H, dd, *J* = 8.4, 1.5), 7.63 (1H, d, *J* = 8.4); ¹³C NMR (CD₃OD) δ 194.2, 146.0, 143.0, 139.8, 133.6, 124.9, 120.7, 116.6; Anal. Calcd for C₈H₆N₂O: C, 65.75; H, 4.14; N, 19.17. Found: C, 65.60; H, 4.17; N, 19.08.

Example 6. 5-Cyano-2-(benzimidazol-5'-yl)benzimidazole (19).

A mixture of 5-formylbenzimidazole 18 (211 mg, 1.44 mmol) and 4-cyano-1,2-phenylenediamine (230 mg, 1.73 mmol) in nitrobenzene (10 ml) was heated at 150°C under N₂ overnight. The mixture was cooled to room temperature and directly chromatographed on silica gel eluting with 0-15% MeOH/EtOAc to give 244 mg (65%) of 18 as a solid: mp >270 °C; IR (KBr) 3110, 2826, 2224, 1627, 1426, 1294; ¹H NMR (CD₃OD) δ 8.41 (1H, s), 8.33 (1H, s), 8.07 (1H, dd, *J* = 8.6, 1.5), 7.98 (1H, s), 7.78 (1H, d, *J* = 8.4), 7.73 (1H, d, *J* = 8.4), 7.56 (1H, dd, *J* = 8.4, 1.5); ¹³C NMR (DMSO-d₆ + 3 drops of CF₃COOH) δ 153.4, 140.4, 138.3, 132.9, 131.6, 127.0, 125.8, 125.3, 120.8, 119.8, 116.0, 115.8, 113.9, 105.5; HRMS (FAB) calcd for C₁₅H₁₀N₅, 260.0936, found 260.0935.

Example 7.

(A) 5-Bromo-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-yl]-benzimidazole (JSK IV-37) A mixture of 5-formyl-2-(benzimidazol-5'-yl)benzimidazole (118.8 mg, 0.45 mmol) and 5-bromophenylenediamine (169.6 mg, 0.90 mmol) in nitrobenzene (5 mL) was heated at 150°C under N₂ overnight. The mixture was cooled to room temperature and chromatographed using 0-10% methanol/ethylacetate to afford 127.3 mg (66%) of brownish yellow solid:

mp>280°C; IR (KBr) 3101, 1626, 1547, 1440; ¹H NMR (DMSO-*d*₆) δ 7.34 (dd, 1H, J=7.0, 2.0), 7.57 (d, 1H, J=9.0), 7.71-7.80 (m, 3H), 8.04-8.18 (m, 2H), 8.39 (s, 2H), 8.50 (s, 1H); ¹³C NMR (DMSO-*d*₆ + 3 drops CF₃COOH) δ 114.1 115.8, 116.2, 116.4, 117.0, 118.6, 123.5, 125.3, 126.2, 128.7, 128.9, 131.8, 132.0, 132.3, 133.1, 134.4, 138.3, 140.6, 151.1, 153.4.

(B) 5-Chloro-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-yl]-benzimidazole (JSK IV-68)

A mixture of 5-formyl-2-(benzimidazol-5'-yl)benzimidazole (160 mg, 0.61 mmol) and 5-chlorophenylenediamine (174 mg, 1.22 mmol) in nitrobenzene (5 mL) was heated at 150°C under N₂ overnight. The mixture was cooled to room temperature and chromatographed using 0-10% methanol/ethylacetate to afford 167 mg (71%) of brownish yellow solid:

mp>280°C; IR (KBr) 3103, 2826, 1427, 1293; ¹H NMR (DMSO-*d*₆) δ 7.24 (dd, 1H, J=8.5, 2.0), 7.60-7.81 (m, 4H), 8.07-8.17 (m, 2H), 8.40 (s, 2H), 8.50 (s, 1H); ¹³C NMR (DMSO-*d*₆ + 3 drops CF₃COOH) δ 114.3, 114.4, 115.3, 115.5, 115.6, 116.2, 118.5, 123.1, 125.4, 125.5, 125.6, 129.4, 132.4, 132.9, 133.0, 135.2, 138.9, 140.9, 151.8, 153.5.

(C) 5-(*p*-Chlorophenyl)- 2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-yl]-

benzimidazole (JSK IV-47) A mixture of 5-formyl-2-(benzimidazol-5'-yl)benzimidazole (99 mg, 0.38 mmol) and 5-(*p*-chlorophenyl)-phenylenediamine (154 mg, 0.71 mmol) in nitrobenzene (5 mL) was heated at 150°C under N₂ overnight. The mixture was cooled to room temperature and chromatographed using 0-10% methanol/ethylacetate to afford 85 mg (49%) of brownish yellow solid: mp>280°C; IR (KBr) 3046, 2820, 1426, 1282; ¹H NMR (DMSO-*d*₆ + 3 drops CF₃COOH) δ 7.56 (d, 2H, J=8.5), 7.82 (d, 2H, J=8.5), 7.88-8.21 (m, 6H), 8.48 (d, 1H, J=8.8), 8.63 (s, 1H) 8.72 (s, 1H), 9.69 (s, 1H); ¹³C NMR (DMSO-*d*₆ + 3 drops CF₃COOH) δ 111.8, 113.8, 114.7, 115.8, 116.1, 117.7, 123.0, 124.1, 125.2, 125.3, 129.2, 129.3, 131.9, 132.1, 133.0, 133.1, 137.2, 138.5, 139.3, 141.6, 150.8, 153.8.

(D) 4-Bromophenylenediamine (JSK IV-35) To 2-nitro-4-bromoaniline (340 mg, 1.57 mmol) in absolute ethanol (20mL) was added SnCl_2 (1.50g, 7.91 mmol) and refluxed overnight. The reaction mixture was then basified to pH 11 with 2N NaOH and extracted with ether to give 275 mg (94%) of product. This product was used without further purification for the synthesis of JSK IV-37.

(E) 4-Chlorophenylenediamine (JSK IV-67) To 2-nitro-5-chloroaniline (304 mg, 1.76 mmol) in absolute ethanol (20 mL) was added SnCl_2 (1.68g, 8.86 mmol) and refluxed overnight. The reaction mixture was then basified to pH 11 with 2N NaOH and extracted with ether to give 250 mg (quantitative yield) of product. This product was used without further purification for the synthesis of JSK IV-68.

(F) *p*-Chlorotributylphenyltin (JSK IV-42) 4-Bromochlorobenzene (3.2 g, 16.62 mmol) was dissolved in dry THF (20mL). After bringing the reaction temperature down to -78°C with an acetone/dry ice bath, $n\text{BuLi}$ (15.58 mL, 1.6M, 1.5 equiv.) was added slowly and stirred at -78°C for 30 min. Tributyltinchloride (6.77 mL, 1.5 equiv.) was added and stirred overnight while bringing the reaction to room temperature. Reaction mixture was quenched by stirring the reaction flask open in air for 1 hour after which THF was rotavaporated off. Product was obtained as an oil (7.35g, 97%) after passing the mixture through a quick silicagel column eluting with 100% hexanes.

(G) 2-Nitro-5-(*p*-chlorophenyl)aniline (JSK IV-44) To JSK IV-42 (2.02 g, 5.04 mmol) and 2-nitro-4-bromoaniline (730 mg, 3.36 mmol) in DMF (18 mL) was added $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (117.9 mg, 0.17 mmol) and PPh_3 (440.2 mg, 1.70 mmol) and heated at 120°C overnight. DMF was rotavaporated off and the mixture was separated on a silicagel column eluting with 5-10% ethylacetate/hexanes to give 270 mg (32%) of reddish solid.

(I) 4-(*p*-Chlorophenyl)phenylenediamine (JSK IV-46) JSK IV-44 (190 mg, 0.77 mmol) was dissolved in ethylacetate (100 mL) and after adding 10% Pd-C (40 mg) was reduced by hydrogenation (45 psi). Product (quantitative yield) was used in JSK IV-47 without further purification.

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Example 8. Bioassays

A. Topoisomerase I-Mediated DNA Cleavage Assays

DNA topoisomerase I was purified from calf thymus gland as reported previously by B. D. Halligan et al., J. Biol. Chem., **260**, 2475 (1985). Plasmid YEpG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described by T. Mariatis et al., Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Labs, NY (1982) at pages 149-185. The end-labeling of the plasmid was accomplished as previously described by L. F. Liu et al., J. Biol. Chem., **258**, 15365 (1983). The cleavage assays were performed as previously reported by A. Y. Chen et al., Cancer Res., **53**, 1332 (1993). Human topoisomerase was isolated as a recombinant fusion protein using a T7 expression system.

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B. Cytotoxicity assay

The cytotoxicity was determined using the as MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA) following the procedures of F. Denizot et al., J. Immunol. Methods, **89**, 271 (1986); J. Carmichael et al., Cancer Res., **47**, 936 (1987) and T. J. Mosmann et al., Immunol. Methods, **65**, 55 (1983). The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan). See, for example, T. Andoh et al., Adv. Pharmacol., **29B**, 93 (1994). The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO₂ and maintained by regular

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passage in RPMI medium supplemented with 10% heat inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (0.1 mg/ml). For determination of IC_{50} , cells were exposed continuously with varying concentrations of drug concentrations and MTT assays were performed at the end of the fourth day.

The drug sensitive human epidermoid carcinoma KB3-1 cell line (S. Aliyama et al., Somatic Cell Mol. Genet., 11, 117 (1985)) and its vinblastine-selected multidrug-resistant variant KBV-1 cells (D. W. Shen et al., Science, 32, 643 (1986)) were provided by Dr. Michael Gottesmann (National Cancer Institute, Bethesda, ML). These cells were grown as monolayer cultures at in 5% CO_2 and maintained by regular passage in Dulbecco's minimal essential medium supplemented with 10% heat inactivated fetal bovine serum. KBV-1 cells were similarly maintained except they were grown in the presence of 1 $\mu g/ml$ vinblastine.

C. Results

As shown on Table 1, comparison of compounds 10-16 with Hoechst 33342 (1) as inhibitors of topoisomerase I demonstrated that several of these trisbenzimidazoles had similar potency.

Table 1.
Topoisomerase I-mediated DNA Cleavage and Cytotoxicity
of Bis- and Trisbenzimidazoles

Compound	Topo I-mediated DNA cleavage ^b	Cytotoxicity IC ₅₀ ^a (μM) <u>Cell Lines</u>			
		RPMI	CPT-K5	KB3-1	KBV-1
Hoechst 33342	1	0.03	0.9	0.01	1.2
10	1.1	14	28	N.D.	N.D.
11	1	> 25 ^c	> 25 ^c	N.D.	N.D.
12	100	7.6	20	N.D.	N.D.
13	2	0.09	0.58	0.58	0.35
14	3.3	0.16	5.8	0.05	0.09
15	2	0.035	2.5	0.02	0.02
16	2	0.035	2.5	0.02	0.01
19	1000	> 25 ^c	N.D.	N.D.	N.D.
JSKIV-37	1	1.40	1.40		
JSKIV-47	10	0.09	0.20		
JSKIV-68	1	1.04	0.65		

a) IC₅₀ has been calculated after 4 days of continuous drug exposure. N.D. = Not determined.

b) Topoisomerase I cleavage values are reported as REC, Relative Effective Concentration, i.e. concentrations relative to Hoechst 33342, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of calf thymus topoisomerase I. Cleavage is calculated from the intensity of the strongest Hoechst specific band.

c) No indication of cytotoxicity were considered indicative of IC₅₀ values substantially greater than the highest doses assayed.

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While **10** and **11** exhibited similar potency in their inhibition of topoisomerase I as observed with Hoechst 33342, both of these compounds failed to exhibit significant cytotoxicity towards the human lymphoblast cell line, RPMI 8402. However, this may be due to the inability of the pure compound to penetrate the target cells, which may be overcome by selection of a suitable carrier, such as liposomes. The 5-phenyl substituted trisbenzimidazole, **13**, was approximately one-half as potent as Hoechst 33342 as a topoisomerase I inhibitor. In contrast to **10** and **11**, however, it had significant cytotoxicity towards the human lymphoblast cell line, RPMI 8402 cells. As observed with Hoechst 33342, **13** was also effective

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against camptothecin-resistant CPT-K5 cells. The relative resistance of Hoechst 33342 and 13, expressed as the ratio of the IC_{50} values of the resistant verses the drug sensitive cell line, is approximately 30 fold as compared to the relative resistance of camptothecin which is 2,500 fold, as reported by A. Y. Chen et al., Cancer Res., 53, 1332 (1993). A similar effect was observed in another pair of cell lines; 13 has an IC_{50} of 0.015 $\mu\text{g/ml}$ in the human ovarian tumor cell line, A2780, relative to an IC_{50} of 0.03 $\mu\text{g/ml}$ in CPT-2000, a variant of A2780 selected for camptothecin-resistance and known to contain a mutant camptothecin-resistant topoisomerase I. The 5-*n*-propyl trisbenzimidazole derivative, 12, was much less active than either 10, 11, or 13 as an inhibitor of topoisomerase I. Its weak activity as a topoisomerase I inhibitor correlated with its weak cytotoxicity. The activity of several of these compounds were also evaluated using recombinant human topoisomerase I. Several of these analogs induced similar DNA cleavage in the presence of human topoisomerase I as compared to that observed with topoisomerase I isolated from calf thymus.

The cytotoxic activity of Hoechst 33342 and 13 was also evaluated against KB 3-1 and KB V-1 cells. The primary difference between these cell lines is in the degree to which human MDR1 (P-glycoprotein) is expressed. Recent studies have demonstrated that antineoplastic agents which are cationic at physiological pH are more likely to serve as substrates for MDR1 and, therefore, are likely to be less effective against cells that overexpress P-glycoprotein. In view of the fact that Hoechst 33342 is extensively protonated at physiological pH, it is not surprising that the IC_{50} differs by approximately two-orders of magnitude for KB 3-1 as compared to KB V-1 cells, as reported by A.Y. Chen et al., Adv. Pharmacol., 245, 29B (1994). In contrast to Hoechst 33342, there is little difference between the IC_{50} values observed for 13 in these two cell lines. Thus, 13 appears not to be a substrate for human MDR1. This data indicate that these trisbenzimidazole derivatives may have significant chemotherapeutic advantages as compared to

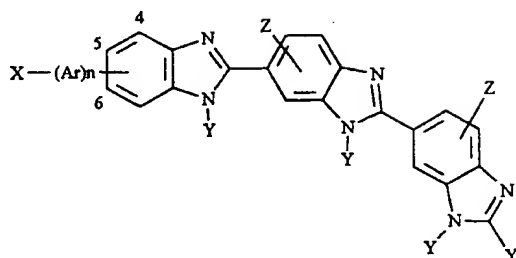
Hoechst 33342 or pibenzimol (Hoechst 33258), 2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole.

These data indicate that substitution of these trisbenzimidazole with a 5-Ar substituent can yield derivatives which are active as topoisomerase I inhibitors and cytotoxic to tumor cells. Trisbenzimidazoles substituted at the 5- position with either a 2-, 3-, or 4-pyridyl group, 14-16, were evaluated for their potency as topoisomerase I inhibitors and for cytotoxicity as summarized in Table 1. These analogs, similar to 13, have activity as topoisomerase I inhibitors. The 3- and 4-pyridyl analogs, 15 and 16, are somewhat more active than the 2-pyridyl derivative, 14, as topoisomerase I inhibitors as well as cytotoxic agents. As was observed with 13, these pyridyl-substituted tribenzimidazoles had similar cytotoxicity to KB 3-1 cells as well as to KB V-1 cells which overexpress MDR1. A principal advantage of these heteroaryl substituted trisbenzimidazoles as compared to Hoechst 33342 is their efficacy against cell lines which express MDR1.

All publications and patents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A compound of the formula:



wherein Ar is (C₆-C₁₂)aryl or (5- to 12-membered)heteroaryl comprising 1-3 N, S or non-peroxide O, wherein N is unsubstituted or is substituted with (C₁-C₄)alkyl, said Ar being optionally fused to the benzo moiety; X is H, CN, CHO, OH, acetyl, CF₃, O(C₁-C₄)alkyl, NO₂, NH₂, halogen or halo-(C₁-C₄)alkyl; each Y is individually H, (C₁-C₄)alkyl or aralkyl; Y' is H or (C₁-C₄)alkyl; each Z is individually H, (C₁-C₄)alkyl, halogen or halo(C₁-C₄)alkyl; and n is 0-1; or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein n is 1 and Ar is at the 5-position.
3. The compound of claim 1 wherein n is 1 and Ar is a 5-6 membered heteroaryl comprising 1-2 N, S or non-peroxide O atoms.
4. The compound of claim 2 wherein Ar is phenyl or pyridyl.
5. The compound of claim 4 wherein pyridyl is 2-pyridyl, 3-pyridyl or 4-pyridyl.

6. The compound of claims 4 or 5 wherein X is H.
7. The compound of claim 6 wherein Y' is H.
8. The compound of claims 4 or 5 wherein each Y is H.
9. The compound of claim 1 wherein n is 0 and X is H, CHO or CN.
10. The compound of claim 9 wherein Y' is H.
11. The compound of claim 4 wherein Ar is phenyl.
12. The compound of claim 11 wherein X is halogen.
13. The compound of claim 12 wherein X is Cl.
14. The compound of claim 13 wherein X-Ar is *p*-chlorophenyl.
15. The compound of claim 14 wherein Y' is H; each Y is H; and each Z is H.
16. The compound of claim 1 wherein n is 0 and X is halogen.
17. The compound of claim 16 wherein X is Cl.
18. The compound of claim 16 wherein X is Br.
19. The compound of claims 17 or 18 wherein Y' is H; each Y is H; and each Z is H.

20. The compound of claim 1 wherein Z is H, F, CH₃ or CF₃.
21. The compound of claim 1 wherein Ar is fused to the benzo moiety.
22. The compound of claim 21 wherein Ar is fused to the 4,5-positions of the benzo moiety.
23. The compound of claim 21 wherein Ar is fused to the 5,6-positions of the benzo moiety.
24. The compound of claim 21 wherein Ar is phenyl.
25. A method of inhibiting the growth of a mammalian tumor cell comprising contacting a tumor cell susceptible thereto, with an effective inhibitory amount of a compound of claim 1, in combination with a pharmaceutically acceptable carrier.
26. A therapeutic composition comprising the compound of claim 1 in combination with a pharmaceutically acceptable carrier.

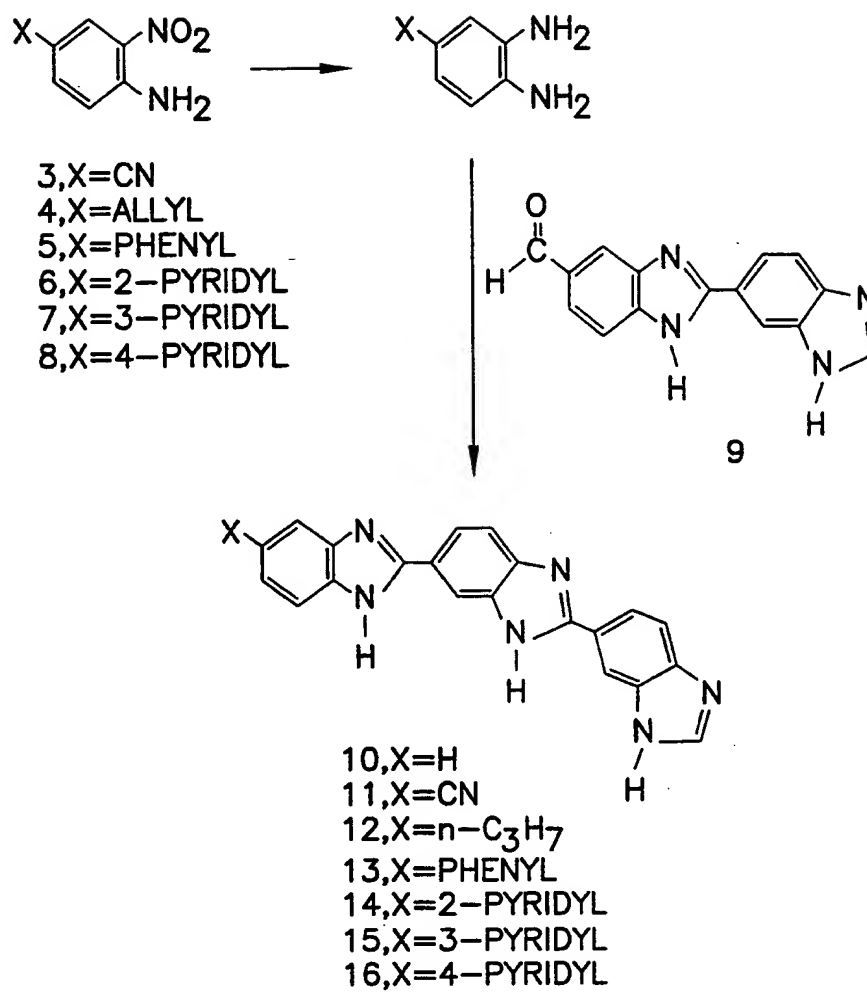


FIG. 1

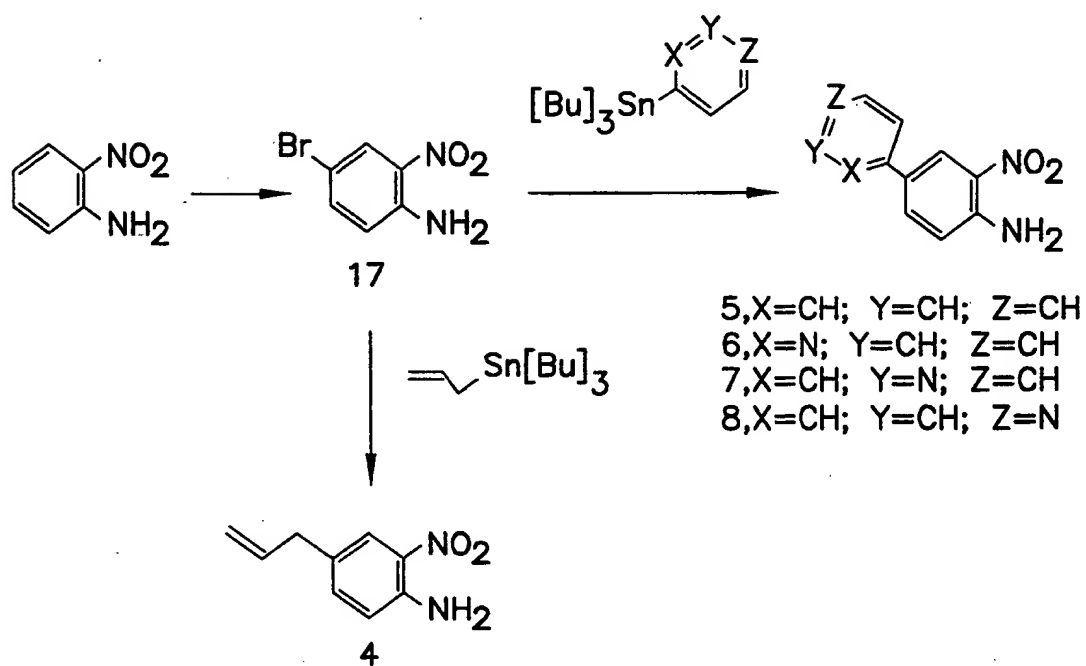


FIG. 2

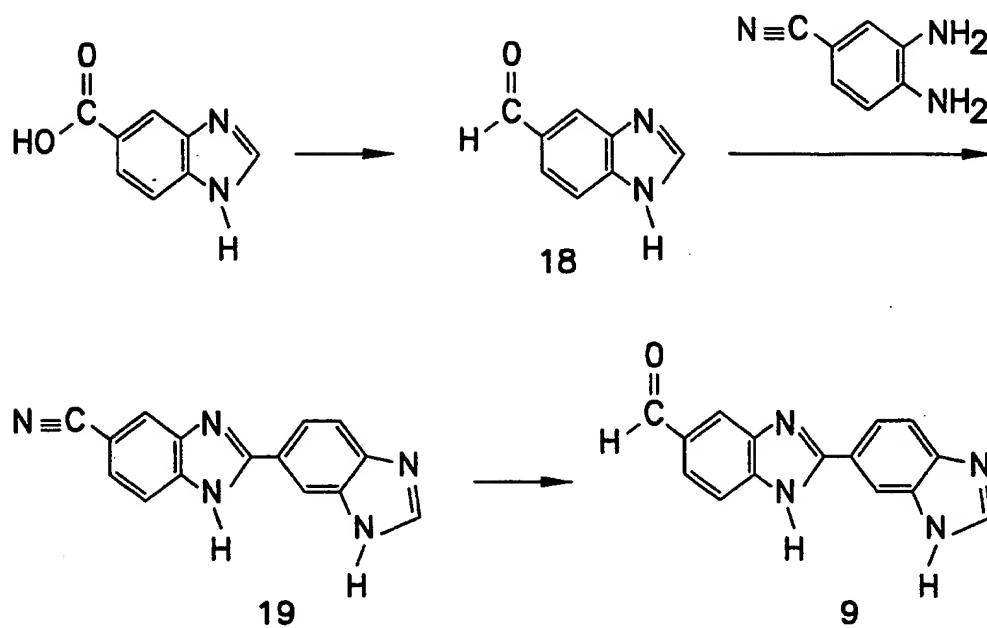
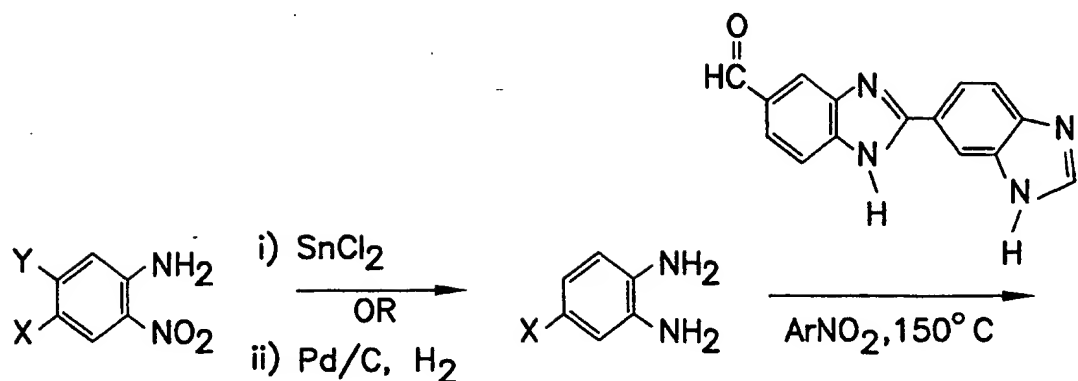
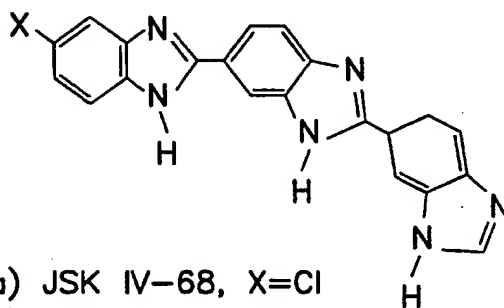


FIG. 3



- a) X=H, Y=Cl
- b) X=Br, Y=H
- c) JSK IV-44

- a) JSK IV-67, i), X=Cl
- b) JSK IV-35, i), X=Br
- c) JSK IV-46, ii), X=p-CHLOROPHENYL



- a) JSK IV-68, X=Cl
- b) JSK IV-37, X=Br
- c) JSK IV-47, X=p-CHLOROPHENYL

FIG. 4

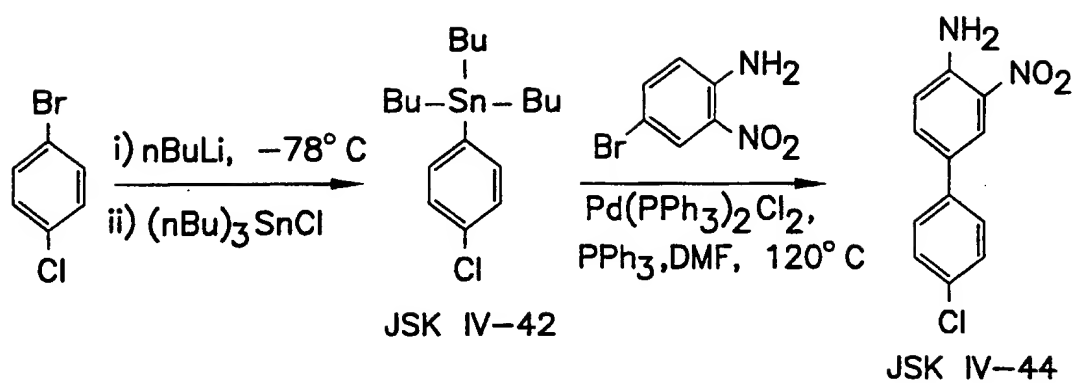


FIG. 5

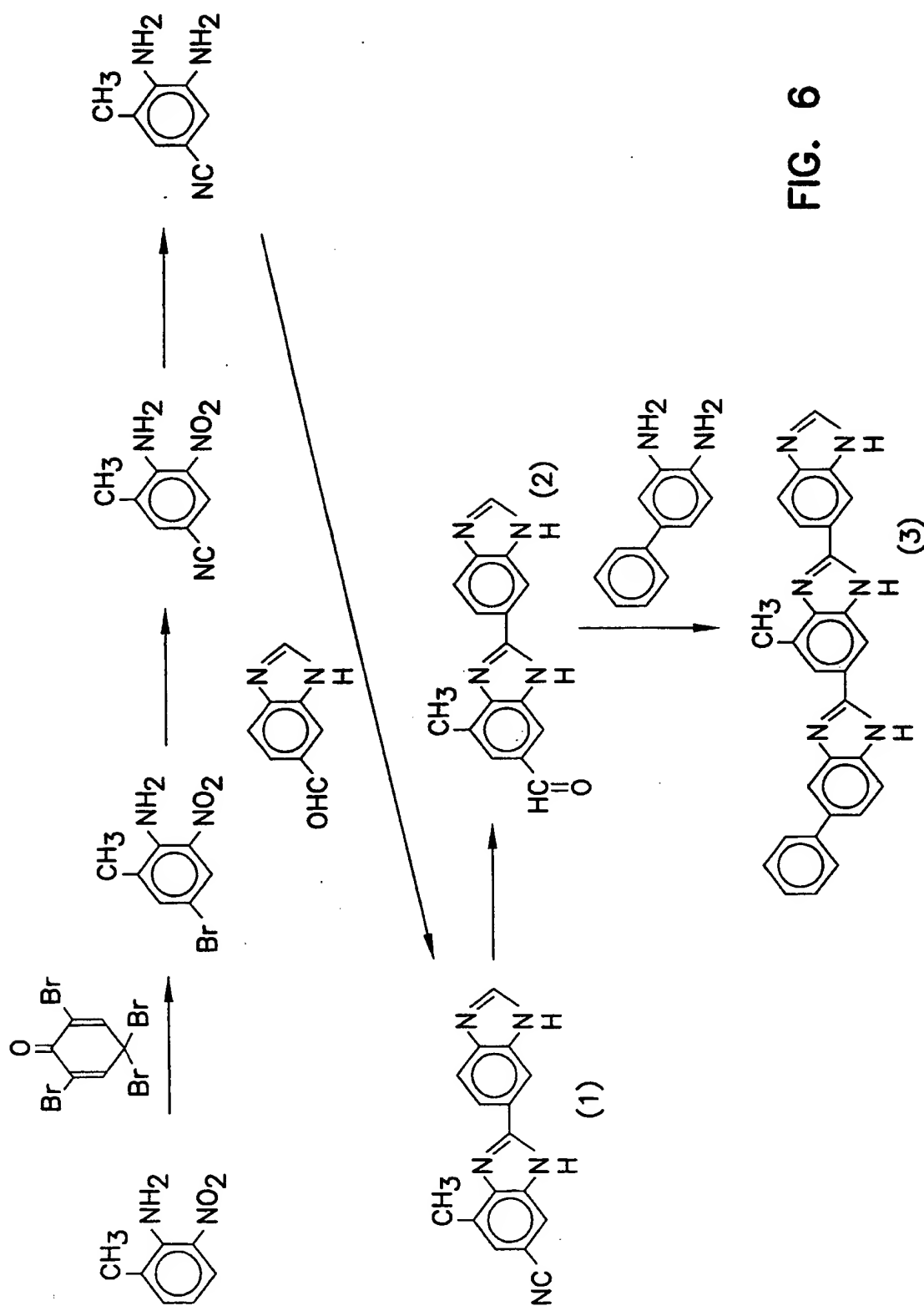
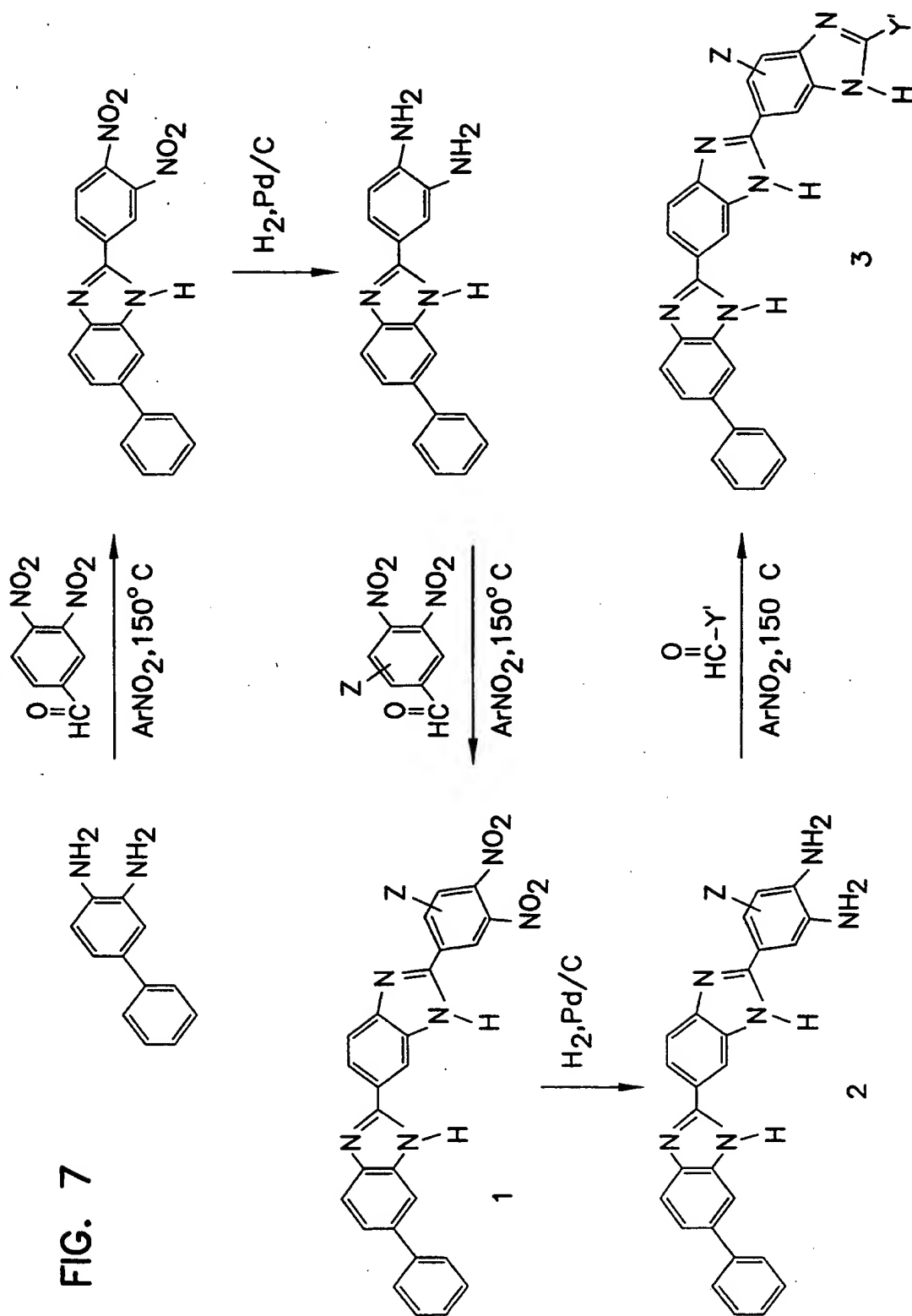


FIG. 6



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/06853

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07D235/18 A61K31/415 C07D401/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CANCER RESEARCH, vol. 53, no. 6, 15 March 1993, MD US, pages 1332-1337, XP002010634 A.Y. CHEN ET AL.: "A New Mammalian DNA Topoisomerase I Poison Hoechst 33342: Cytotoxicity and Drug Resistance in Human Cell Cultures" cited in the application see the whole document ---	1-26
P,X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 38, no. 18, 1 September 1995, WASHINGTON US, pages 3638-3644, XP002010635 Q. SUN ET AL.: "Synthesis and Evaluation of Terbenzimidazoles as Topoisomerase I Inhibitors" see the whole document -----	1-26

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

9 August 1996

Date of mailing of the international search report

19. 08. 96

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Authorized officer

Fink, D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 06853

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 25 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.